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Natriuretic peptide receptors mediate different responses in rat renal microvessels

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Natriuretic peptide receptors mediate different responses in rat renal microvessels. Atrial natriuretic peptide (ANP) has unique effects on the renal vasculature, in that it dilates preglomerular vessels and constricts efferent arterioles. In the present study we aimed to characterize the natriuretic peptide receptor (NPR) subtypes, which mediate the renovascular effects of ANP, using *in vivo* microscopy in the split hydronephrotic kidney model of rats. ANP (10^{-9} and $3 \cdot 10^{-9}$), which binds to NPR-A and NPR-C, dilated preglomerular vessels and constricted efferent arterioles similarly to that found in previous studies. C-type natriuretic peptide (10^{-9} to 10^{-7}), which binds to NPR-B and NPR-C, dilated pre- and postglomerular vessels and profoundly increased glomerular blood flow. A specific ligand of NPR-C, C-ANP (des-[Gln^{18},Ser^{19},Gly^{20},Leu^{21},Gly^{22}]ANP$_{4\text{-}23}$-NH$_2$, 10^{-9} to 10^{-7}) was devoid of vascular effects. The ANP antagonist A71915 (10^{-9} to 10^{-6}) induced moderate dilation in renal vessels possibly due to some agonistic activity on NPR-B. ANP-induced preglomerular vasodilation was attenuated by A71915 (10^{-6}) to 36 ± 6% of the initial response, whereas efferent vasoconstriction was completely abolished (−4 ± 4% of initial response). Our results indicate that ANP dilates preglomerular vessels and constricts efferent arterioles through NPR-A, both responses being antagonized by A71915 with different potencies. Furthermore, our data show that in the rat renal microcirculation stimulation of NPR-B results in vasodilation only, whereas NPR-C does not mediate vascular responses.

Since the discovery of atrial natriuretic peptide (ANP) in 1981 [1], it has been shown that inhibition of tubular sodium reabsorption and an increase in glomerular filtration rate are involved in the natriuresis induced by this hormone [2]. The increase in glomerular filtration rate is due to an increase in glomerular filtration pressure [3]. This increase in pressure is the result of preglomerular vasodilation and efferent arteriolar constriction, both of which are mediated by ANP [4, 5]. Currently, three subtypes of natriuretic peptide receptors (NPR) are known [6, 7]. Most of the biological effects are thought to be mediated via NPR-A and NPR-B, which possess intrinsic guanylyl cylase activity [8], while NPR-C was proposed to serve mainly as a clearance receptor [9]. ANP administration results in vasodilation, a decrease in blood pressure, and natriuresis. These responses have been attributed to stimulation of NPR-A, the high affinity binding site of ANP [10], and the subsequent increase in intracellular cGMP [2]. In contrast to this understanding of the preglomerular response, it is unclear by which receptor and mechanism ANP induces constriction in the efferent arteriole [11].

The important role of ANP gene products in extracellular volume regulation has recently been underlined by the observation of salt-sensitive hypertension in mice with a disruption of the proANP gene [12]. However, disruption of the NPR-A gene in mice leads to chronic elevation of blood pressure, which is unexpectedly not influenced by salt intake [13]. This finding suggests that receptors other than NPR-A are involved in the natriuretic action of ANP. In addition, a class of ANP antagonists was developed by von Geldern and coworkers, which antagonized the natriuretic effects but not the vasorelaxant and hypotensive effects of ANP [14]. The aim of our present study was therefore to characterize the NPR subtypes involved in the ANP-induced renal vascular responses. As a model for the renal microcirculation we used the split hydronephrotic rat kidney [15], which exhibits a comparable vascular response to ANP [4, 11] as in the normal rat kidney [3, 5]. In the absence of systemic side effects, we examined the local, vascular effects of the ANP antagonist A71915 [14], and of ANP, C-type natriuretic peptide (CNP) and C-ANP, which are specific ligands for NPR-A, NPR-B and NPR-C, respectively [9, 10].

METHODS

Preparation of the hydronephrotic kidney

Experiments were performed on female Wistar rats (body wt 258 ± 30 g, mean ± SD) in accordance with official guidelines for animal protection. The technique of splitting the rat kidney has been previously described in detail [15]. In brief, ligation of the ureter via a flank incision was performed during pentobarbital sodium anesthesia (Nembutal® 60 mg · kg^{-1} i.p.; Ceva, Bad Segeberg, Germany). The final experiments were performed under thiobutabarbital anesthesia (Inactin® 100 mg · kg^{-1} i.p.; Byk Gulden, Konstanz, Germany) about three months after induction of hydronephrosis. Body temperature was maintained at 37.0 to 37.5°C via a heating table, systemic blood pressure was monitored via a cannula in the left femoral artery, and isotonic saline (3 ml · hr^{-1}) was continuously infused via a cannula placed in the jugular vein. After exposure of the left hydronephroptic kidney by a flank incision, the kidney was split along the greater curvature with a thermal cautery. The ventral half of the kidney was sutured to a semicircular wire frame that was attached to the bottom of a plexiglass bath. The entry of the renal hilus into the bath was sealed with silicone grease, and the bath was filled with

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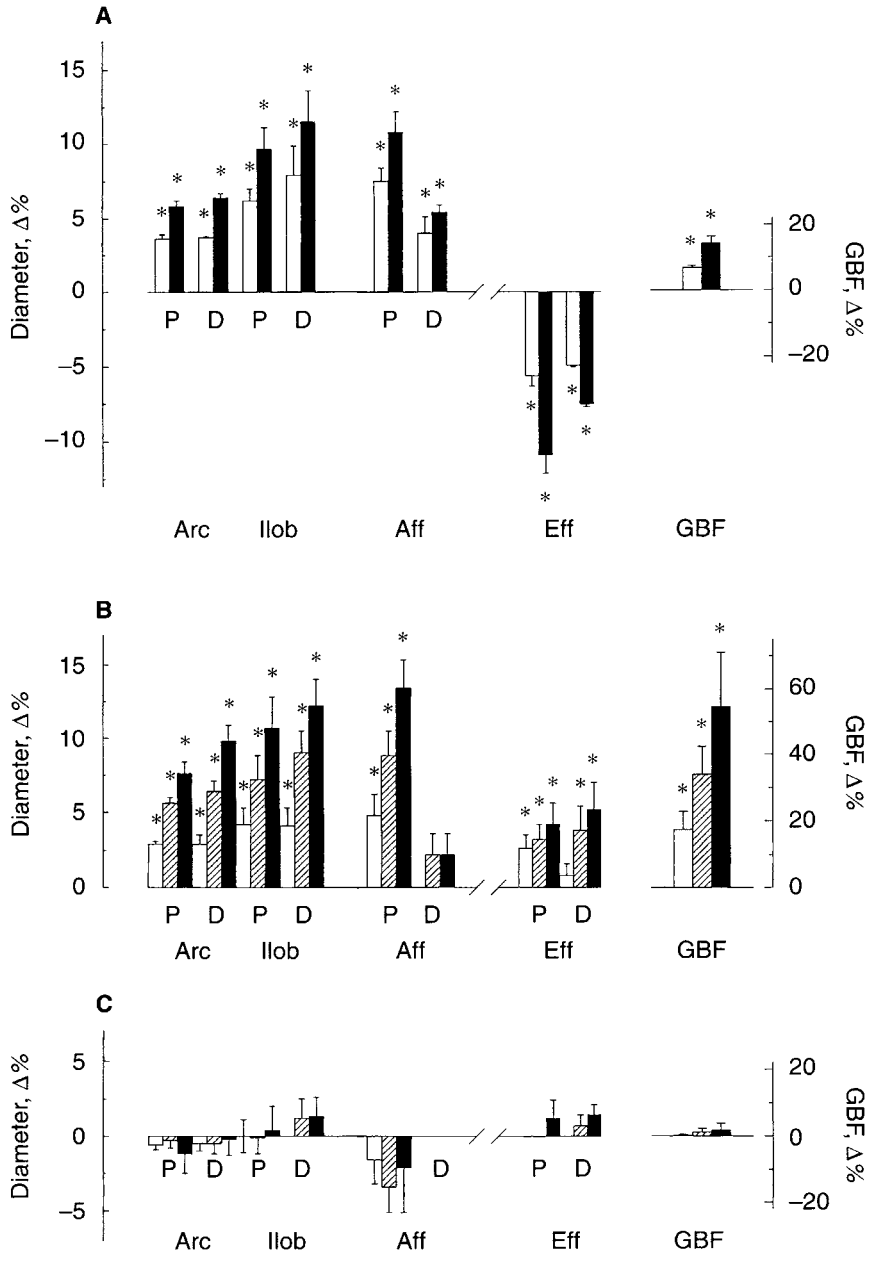

Fig. 1. Percent changes of renal vascular diameters and of GBF in response to ANP (*A*), CNP (*B*), and C-ANP (*C*). Symbols in A are: (□) 10^{-9}; (■) $3 \cdot 10^{-9}$ ANP ($N = 5$). Symbols in B are: (□) 10^{-9}; (▨) 10^{-8}; (■) 10^{-7} CNP ($N = 5$). In C, symbols are: (□) 10^{-9}; (▨)10^{-8}; (■) 10^{-7} C-ANP ($N = 3$). Data are means ± SEM. *$P < 0.05$ versus control. Abbreviations are: arc, arcuate artery; ilob, interlobular artery; aff, afferent arteriole; eff, efferent arteriole; p, proximal; d, distal.

an isotonic, isocolloidal solution (Haemaccel®; Behringwerke, Marburg/Lahn, Germany) maintained at 37°C. A Leitz Ultropak water immersion objective (UO-55) was combined with a television and video recording system for *in vivo* transillumination microscopy. Kidney preparations were allowed to equilibrate in the tissue bath for at least one hour after the surgical procedure. The microcirculatory parameters of this preparation have been demonstrated to be stable for more than three hours [16].

Measurements

Renal vascular segments. Vessels were identified according to their branching pattern from a selected glomerulus. Lumen diameters of the following vascular segments were measured (abbreviations serve for identification in Figs. 1 and 2): proximal arcuate artery, near the interlobar artery (arc, p); distal arcuate artery, near the interlobular artery (arc, d); proximal interlobular artery, near the arcuate artery (ilob, p); distal interlobular artery, near the afferent arteriole (ilob, d); proximal afferent arteriole, near the interlobular artery (aff, p); distal afferent arteriole, at the narrowest segment before entering the glomerulus (aff, d); proximal efferent arteriole, within 50 μm of the glomerulus (eff, p); distal efferent arteriole, near the first branching point (eff, d).

Glomerular blood flow (GBF). Red blood cell velocity was determined in the efferent arteriole by a velocity tracking correlator (Model 102B; IPM Inc., San Diego, CA, USA) [17]. To obtain GBF, the measured red cell velocity was multiplied by the luminal cross section of the efferent arteriole and corrected for the Fahraeus effect [18].

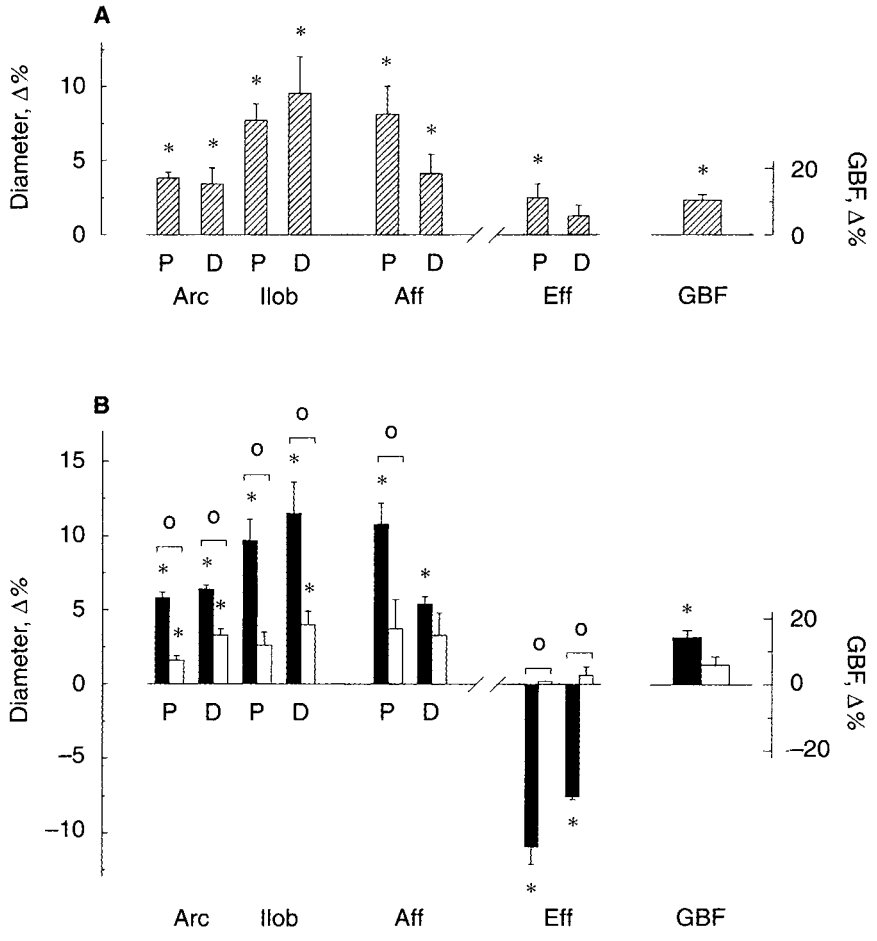

Fig. 2. Percent changes of renal vascular diameters and of GBF in response to the ANP antagonist A71915 (*A*) and to ANP in the absence or presence of A71915 (*B*). Symbols are: (▨) 10^{-6} A71915 (N = 8); $3 \cdot 10^{-9}$ ANP in the absence (■) or presence (□) of 10^{-6} A71915 (N = 5). Data are means ± SEM of percent changes versus control (hatched and filled columns) or versus 10^{-6} A71915 (open columns), $^{*}P < 0.05$ versus control (hatched and filled columns) or versus 10^{-6} A71915 (open columns), $^{\circ}P < 0.05$ significant attenuation of ANP response by A71915.

Experimental protocol

Each protocol started with two control periods separated by 10 minutes. Each protocol was made up of several periods, at the end of which systemic blood pressure, GBF, and vascular diameters were measured. All drugs were given into the kidney tissue bath.

Series 1 (N = 5), ANP and A71915 + ANP. ANP (kindly provided by W.-G. Forssmann, Lower Saxony Institute for Peptide Research, Hannover, Germany) was applied in two concentrations (10^{-9} and $3 \cdot 10^{-9}$) each for 15 minutes. Thereafter, ANP was washed out by exchanging the tissue bath three times in 10 minute intervals. Hydronephrotic kidneys were then exposed to increasing concentrations (10^{-9}, 10^{-8}, 10^{-7}, and 10^{-6}) of the ANP antagonist A71915 [14] (kindly provided by Abbott Laboratories, Abbott Park, IL, USA) in 15 minute periods. In the presence of 10^{-6} A71915, ANP was added with the same protocol as at the beginning. Measurements were performed at the end of each 15 minute period and after washout.

Series 2 (N = 3), A71915 + ANP. The last control measurement was followed by application of A71915 and subsequent addition of ANP with the same protocol as in series 1.

Series 3 (N = 5), CNP. Increasing concentrations (10^{-9}, 10^{-8}, and 10^{-7}) of CNP_{1-22} (Bachem, Heidelberg, Germany) were given into the tissue bath. Kidneys were exposed to each concentration for 15 minutes. Measurements were done at the end of each 15 minute period.

Series 4 (N = 3), C-ANP. C-ANP (des-[Gln^{18},Ser^{19},Gly^{20},Leu^{21},Gly^{22}]ANP_{4-23}-NH_2; Sigma, Deisenhofen, Germany), a specific ligand for NPR-C [9], was applied in increasing concentrations (10^{-9}, 10^{-8}, and 10^{-7}) with the same protocol as in series 3.

Data analysis

All values are presented as mean ± SEM. Changes in vascular diameters and GBF are expressed as percentage changes from the last control values unless otherwise stated. Analysis of variance, the Bonferroni method for multiple comparisons, and paired and unpaired t-tests were used as appropriate to test for statistical significance. The overall significance level was set to $P < 0.05$.

RESULTS

The control values of vessel diameters, GBF, blood pressure, and body wt are summarized in Table 1. Blood pressure did not significantly change in any series of experiments.

ANP. ANP, which binds to NPR-A and NPR-C, concentration-dependently dilated preglomerular vessels and constricted efferent arterioles (Fig. 1A). The maximal dilation at $3 \cdot 10^{-9}$ ANP was observed in distal interlobular arteries (12 ± 2%) and proximal afferent arterioles (11 ± 1%); the maximal constriction was located in the proximal part of the efferent arteriole (11 ± 1%). Due to rather equal but opposite effects of ANP on pre- and

Table 1. Control values of vessel lumen diameters, GBF, blood pressure, and body weight

			Series 1 ($N = 5$)	Series 2 ($N = 3$)	Series 3 ($N = 5$)	Series 4 ($N = 3$)
Arcuate artery	proximal	μm	59.2 ± 8.3	69.9 ± 5.2	58.5 ± 9.6	56.3 ± 9.0
	distal	μm	44.0 ± 3.0	48.5 ± 2.6	39.2 ± 7.0	39.0 ± 5.6
Interlobular artery	proximal	μm	21.8 ± 1.4	24.0 ± 2.2	21.5 ± 2.3	23.9 ± 3.7
	distal	μm	14.8 ± 2.3	14.3 ± 0.9	12.7 ± 3.3	12.9 ± 3.6
Afferent arteriole	proximal	μm	11.1 ± 1.5	10.5 ± 0.5	9.3 ± 1.4	9.3 ± 1.2
	distal	μm	9.1 ± 1.4	7.1 ± 1.7	7.5 ± 1.9	7.7 ± 0.5
Efferent arteriole	proximal	μm	15.5 ± 2.4	14.3 ± 1.8	15.2 ± 4.8	15.0 ± 2.3
	distal	μm	18.4 ± 1.1	19.2 ± 3.9	20.4 ± 3.2	20.3 ± 2.4
GBF $nl \cdot min^{-1}$			79 ± 5	55 ± 21	37 ± 21	27 ± 10
Blood pressure *mm Hg*			112 ± 5	110 ± 5	108 ± 10	105 ± 5
Body weight *g*			272 ± 19	253 ± 42	254 ± 35	243 ± 6

Data are means ± SD.

postglomerular vessels, the increase in GBF was scant (14 ± 2% at $3 \cdot 10^{-9}$). After the washout of ANP, all parameters returned to baseline.

CNP. CNP, which binds to NPR-B and NPR-C, concentration-dependently dilated renal vessels and increased GBF (Fig. 1B). Vasodilation and GBF increase were already significant at 10^{-9} CNP. Maximal dilation at 10^{-7} CNP was observed in distal interlobular arteries (12 ± 2%) and proximal afferent arterioles (13 ± 2%). Efferent arteriolar dilation was less pronounced in response to CNP (about 5% at 10^{-7}). Pre- and postglomerular dilation resulted in a clear increase in GBF (55 ± 17% at 10^{-7}).

C-ANP. Local application of C-ANP (10^{-9} up to 10^{-7}), a specific ligand of NPR-C, did not significantly affect diameters of preglomerular vessels and efferent arterioles (Fig. 1C). GBF remained unchanged.

A71915 and ANP. The ANP antagonist A71915 induced identical dilation of renal vessels, irrespective of whether A71915 was given directly after the control measurements (series 2) or after the washout of ANP (series 1). The results of A71915 obtained in series 1 and 2 were therefore pooled together ($N = 8$; Fig. 2A). The dilation in some vessels and the GBF increase became already significant at 10^{-8} A71915 (data not shown). At 10^{-6}, A71915 significantly dilated all preglomerular vessels (3 to 10%). In the efferent arteriole, A71915 had no effects on the distal part and induced slight dilation in the proximal part.

A71915 (10^{-6}) significantly antagonized the effects of ANP in series 1 (Fig. 2B). In the presence of A71915, ANP-induced ($3 \cdot 10^{-9}$) preglomerular dilation was attenuated to 36 ± 6% of the initial response. ANP-induced constriction of the efferent arteriole was completely abolished by A71915 (−4 ± 4% of the initial response, $P < 0.001$ vs. preglomerular attenuation). In series 2, the effects of ANP in the presence of A71915 did not differ from those in series 1.

DISCUSSION

ANP induced preglomerular vasodilation and constriction in efferent arterioles in a concentration-dependent manner similar as in our previous studies [4, 11]. These unique effects of ANP on the renal vasculature, which have also been demonstrated in rat isolated afferent and efferent arterioles [5] and at the whole-kidney level by micropuncture in the normal rat kidney [3], mediate the increase in GFR and thus contribute to ANP-induced natriuresis [2]. At nanomolar concentrations, ANP binds to NPR-A and NPR-C only [10]. Since the NPR-C ligand C-ANP was devoid of vascular effects, preglomerular vasodilation was likely to be caused via NPR-A. Consistent with our finding, Ardaillou and coworkers showed in cultured vascular smooth muscle cells from the rabbit renal cortex, that ANP increases cGMP also via NPR-A with a threshold concentration of 10^{-11} [19], which is in the range of normal circulating ANP concentrations of 1 to $5 \cdot 10^{-11}$ [2]. However, it has to be mentioned that several reports seriously question the role of cGMP in ANP-induced vasodilation [20].

In contrast to preglomerular vasodilation, the mechanism of efferent arteriolar constriction in response to ANP is poorly understood. Since both CNP and C-ANP failed to constrict efferent arterioles, NPR-B and NPR-C appear not to be involved in ANP-induced constriction in our experiments. Furthermore, the ability of A71915 to antagonize the effects of ANP on both preglomerular vessels and efferent arterioles indicates that ANP induced efferent vasoconstriction via binding to NPR-A. In a previous study, urodilatin-induced efferent vasoconstriction was abolished after bradykinin B_2 or endothelin $ET_{A/B}$ receptor blockade, and was enhanced after nitric oxide synthase inhibition [11]. Since urodilatin and ANP share similar NPR binding properties, the present results could imply that stimulation of (endothelial?) NPR-A decreases local bradykinin, and increases local endothelin and nitric oxide concentrations. However, the actual mechanism of the interaction between NPR-A stimulation and these autacoids remains unclear.

A71915 belongs to a class of compounds, which were developed to inhibit ANP-induced cGMP production with weak intrinsic stimulation in bovine transformed aortic endothelial cells and in rat aortic vascular smooth muscle cells [14]. 10^{-5} A71915 caused a 2.8-log shift in the ANP dose-response curve in these assays and inhibited cGMP production with a pA_2 value of 9.2 in a neuroblastoma cell line that exclusively contains NPR-A [21]. In our experiments A71915 itself caused distinct vasodilation, the pattern of which was similar to that of CNP and could therefore reflect some agonistic activity of A71915 at NPR-B. Similar vasodilating properties have been reported for A74186, which was examined in more detail as a representative compound of this class of antagonists [14]. A74186 relaxed rabbit aortic rings *in vitro* and decreased blood pressure in the rat *in vivo*.

Though A71915 completely blocked ANP-induced efferent vasoconstriction, preglomerular vasodilation was only partially

antagonized. Due to the intrinsic vasodilating properties of A71915 in preglomerular vessels, A71915 might even be a weaker antagonist for ANP-induced vasodilation as it is suggested by our results, since ANP-induced vasodilation is diminished unspecifically after reduction of vascular tone by vasodilators [11]. Similar to our results, A74186 hardly antagonized ANP-induced hypotension *in vivo*, whereas it abolished ANP-induced natriuresis [14]. The existence of distinct ANP receptors for mediating vasorelaxation and natriuresis has been proposed to explain the salt-insensitive hypertension in NPR-A knock-out mice [13]. In addition, different mechanisms for the effects of ANP on pre- and postglomerular vessels were suggested by our previous finding, showing that pre- and postglomerular vascular effects of ANP are differently modulated by vasoactive hormones [11].

CNP, besides binding to NPR-C, is a specific ligand for NPR-B at the concentrations we used [10]. CNP relaxed preconstricted rat aorta by virtue of binding to NPR-B [22]. In our experiments, CNP induced overall vasodilation indicating the presence of NPR-B in renal vessels. Binding studies in isolated preglomerular vessels of rats, however, failed to detect NPR-B [23]. On the other hand, the presence of NPR-B mRNA in the renal vasculature has been confirmed by polymerase chain reaction [24]. Presently, renal blood flow in response to CNP has been measured only in dogs [25]. In this study, CNP neither affected renal blood flow nor renal sodium excretion. However, currently available data on CNP suggest that the renal effects may be species-dependent, since CNP was antinatriuretic in another study on dogs [26], whereas it induced natriuresis in the sheep and in the rat at comparable doses [27, 28].

C-ANP has been introduced as a specific ligand for NPR-C [9]. Using C-ANP, it has been shown that NPR-C mainly serves as a clearance receptor accepting all natriuretic peptides with high affinity [9, 10]. In our experiments, C-ANP was devoid of vascular effects at concentrations, which have been reported to saturate NPR-C in renal vessels [23]. Thus, our results support the concept of a biologically silent NPR-C. In agreement with our finding, C-ANP did also not affect vascular resistance and GFR in the isolated perfused rat kidney [9]. Blockade of NPR-C *in vivo* diminishes the clearance of circulating natriuretic peptides, resulting in cardiovascular effects that are similar to those in response to exogenous ANP application [9]. However, local application of C-ANP in the hydronephrotic kidney blocks only a small fraction of totally available NPR-C in the animal. Therefore, local application of C-ANP should not affect the clearance and the levels of natriuretic peptides, which is consistent with our results.

In addition to the concept of NPR-C as a clearance receptor, there are several reports suggesting that NPR-C could also mediate biological effects [20, 29–31]. In almost all of these studies, C-ANP behaved as an agonist for the NPR-C-mediated effects [29–31]. In one study, however, C-ANP antagonized NPR-C-mediated antiproliferative effects on smooth muscle cells [32]. Moreover, it has been proposed that C-ANP binding sites might represent a heterogeneous receptor population [23, 31]. In a study using isolated rat glomeruli, one NPR-C population internalized ANP corresponding to the clearance receptor, whereas another population of NPR-C mediated a decrease in cAMP [31]. Since A71915 also contains the peptide sequence, Arg^{11}, Ile^{12}, Asp^{13}, Arg^{14}, Ile^{15}, which is the minimal structural requirement for NPR-C binding [7], we cannot exclude, however, the remote possibility that ANP constricts efferent arterioles via a subclass of NPR-C, on which both A71915 and C-ANP act as antagonists and which is a low affinity binding site for CNP at the same time.

In the present study we used the split hydronephrotic kidney, an established model for the rat renal microcirculation [33]. Besides obvious alterations–the hydronephrotic kidney is a non-filtering kidney devoid of tubular structures–kidney-specific vascular responses are well preserved in this model, in particular for ANP [34]. Furthermore, local application of NPR ligands permitted to assess their renal vascular effects in the absence of otherwise substantial systemic effects.

In summary, our results show that NPR-A and NPR-B mediate renal vascular responses in the rat, while the gross function of NPR-C is consistent with the concept of a clearance receptor on renal vessels. While stimulation of NPR-B resulted in vasodilation only, NPR-A appears to mediate both preglomerular vasodilation and efferent arteriolar constriction in response to ANP. The ANP antagonist A71915 antagonized pre- and postglomerular effects of ANP, however, with different potencies.

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REFERENCES

1. De Bold AJ, Borenstein HB, Verres AT, Sonnenberg H: A rapid and potent natriuretic response to intravenous injection of atrial myocardial extract in rats. *Life Sci* 28:89–94, 1981
2. Atlas SA, Maack T: Atrial natriuretic factor, in *Handbook of Physiology*, edited by Windhager EE, New York, Oxford University Press, 1992
3. Dunn BR, Ichikawa I, Pfeffer JM, Troy JL, Brenner BM: Renal and systemic hemodynamic effects of synthetic atrial natriuretic peptide in the anesthetized rat. *Circ Res* 59:237–246, 1986
4. Marin-Grez M, Fleming JT, Steinhausen M: Atrial natriuretic peptide causes pre-glomerular vasodilatation and post-glomerular vasoconstriction in rat kidney. *Nature* 324:473–476, 1986
5. Lanese DM, Yuan BH, Falk SA, Conger JD: Effects of atriopeptin III on isolated rat afferent and efferent arterioles. *Am J Physiol* 261:F1102–F1109, 1991
6. Koller KJ, Goeddel DV: Molecular biology of the natriuretic peptides and their receptors. *Circulation* 86:1081–1088, 1992
7. Maack T: Receptors of atrial natriuretic factor. *Annu Rev Physiol* 54:11–27, 1992
8. Drewett JG, Garbers DL: The family of guanylyl cyclase receptors and their ligands. *Endocrine Rev* 15:135–162, 1994
9. Maack T, Suzuki M, Almeida FA, Nussenzveig D, Scarborough RM, McEnroe GA, Lewicki JA: Physiological role of silent receptors of atrial natriuretic factor. *Science* 238:675–678, 1987
10. Suga SI, Nakao K, Hosoda K, Mukoyama M, Ogawa Y, Shirakami G, Arai H, Saito Y, Kambayashi Y, Inouye K, Imura H: Receptor selectivity of natriuretic peptide family, atrial natriuretic peptide, brain natriuretic peptide and C-type natriuretic peptide. *Endocrinology* 130:229–239, 1992
11. Endlich K, Forssmann WG, Steinhausen M: Effects of urodilatin in the rat kidney: Comparison with ANF and interaction with vasoactive substances. *Kidney Int* 47:1558–1568, 1995
12. John SWM, Krege JH, Oliver PM, Hagaman JR, Hodgin JB, Pang SC, Flynn TG, Smithies O: Genetic decreases in atrial

natriuretic peptide and salt-sensitive hypertension. *Science* 267:679–681, 1995
13. LOPEZ MJ, WONG SKF, KISHIMOTO I, DUBOIS S, MACH V, FRIESEN J, GARBERS DL, BEUVE A: Salt-resistant hypertension in mice lacking the guanylyl cyclase-A receptor for atrial natriuretic peptide. *Nature* 378:65–68, 1995
14. VON GELDERN TW, BUDZIK GP, DILLON TP, HOLLEMAN WH, HOLST MA, KISO Y, NOVOSAD EI, OPGENORTH TJ, ROCKWAY TW, THOMAS AM, YEH S: Atrial natriuretic peptide antagonists: Biological evaluation and structural correlations. *Mol Pharmacol* 38:771–778, 1990
15. STEINHAUSEN M, SNOEI H, PAREKH N, BAKER R, JOHNSON PC: Hydronephrosis: A new method to visualize vas afferens, efferens, and glomerular network. *Kidney Int* 23:794–806, 1983
16. STEINHAUSEN M, BLUM M, FLEMING JT, HOLZ FG, PAREKH N, WIEGMAN DL: Visualization of renal autoregulation in the split hydronephrotic kidney of rats. *Kidney Int* 35:1151–1160, 1989
17. WAYLAND H, JOHNSON PC: Erythrocyte velocity measurement in microvessels by a two-slit photometric method. *J Appl Physiol* 22:333–337, 1967
18. GAEHTGENS P, BENNER KU, SCHICKENDANTZ S, ALBRECHT KH: Method for simultaneous determination of red cell and plasma flow velocity in vitro and in vivo. *Pflügers Arch* 361:191–195, 1976
19. BÉA ML, DUSSAULE JC, BENS M, ARDAILLOU R: Characterization of ANF receptors in cultured renal cortical vascular smooth muscle cells. *Am J Physiol* 260:C424–C432, 1991
20. ANAND-SRIVASTAVA MB, TRACHTE GJ: Atrial natriuretic factor receptors and signal transduction mechanisms. *Pharmacol Rev* 45:455–497, 1993
21. DELPORTE C, WINAND J, POLOCZEK P, VON GELDERN T, CHRISTOPHE J: Discovery of a potent natriuretic peptide antagonist for ANP_A receptors in the human neuroblastoma NB-OK-1 cell line. *Eur J Pharmacol* 224:183–188, 1992
22. DREWETT JG, FENDLY BM, GARBERS DL, LOWE DG: Natriuretic peptide receptor B (guanylyl cyclase-B) mediates C-type natriuretic peptide relaxation of precontracted rat aorta. *J Biol Chem* 270:4668–4674, 1995
23. DE LEON H, BONHOMME MC, GARCIA R: Rat renal preglomerular vessels, glomeruli and papillae do not express detectable quantities of B-type natriuretic peptide receptor. *J Hypertens* 12:539–548, 1994
24. TEREDA Y, TOMITA K, NONOGUCHI H, YANG T, MARUMO F: PCR localization of C-type natriuretic peptide and B-type receptor mRNAs in rat nephron segments. *Am J Physiol* 267:F215–F222, 1994
25. CLAVELL AL, STINGO AJ, WEI CM, HEUBLEIN DM, BURNETT JC JR: C-type natriuretic peptide: A selective cardiovascular peptide. *Am J Physiol* 264:R290–R295, 1993
26. STINGO AJ, CLAVELL AL, AARHUS LL, BURNETT JC JR: Cardiovascular and renal actions of C-type natriuretic peptide. *Am J Physiol* 262:H308–H312, 1992
27. CHARLES CJ, ESPINER EA, RICHARDS AM, NICHOLLS MG, YANDLE TG: Biological actions and pharmacokinetics of C-type natriuretic peptide in conscious sheep. *Am J Physiol* 268:R201–R207, 1995
28. KOMEICHI H, MOREAU R, CAILMAIL S, GAUDIN C, LEBREC D: Blunted natriuresis and abnormal systemic hemodynamic responses to C-type and brain natriuretic peptides in rats with cirrhosis. *J Hepatol* 22:319–325, 1995
29. LEVIN ER, FRANK HJL: Natriuretic peptides inhibit rat astroglial proliferation: Mediation by the C receptor. *Am J Physiol* 261:R453–R457, 1991
30. DAI LJ, QUAMME GA: Atrial natriuretic peptide initiates Ca^{2+} transients in isolated renal cortical thick ascending limb cells. *Am J Physiol* 265:F592–F597, 1993
31. BROWN J, ZUO Z: Receptor proteins and biological effects of C-type natriuretic peptide in renal glomerulus of the rat. *Am J Physiol* 266:R1383–R1394, 1994
32. CAHILL PA, HASSID A: Clearance receptor-binding atrial natriuretic peptides inhibit mitogenesis and proliferation of rat aortic smooth muscle cells. *Biochem Biophys Res Commun* 179:1606–1613, 1991
33. NAVAR LG, INSCHO EW, MAJID DSA, IMIG JD, HARRISON-BERNARD LM, MITCHELL KD: Paracrine regulation of the renal microcirculation. *Physiol Rev* 76:425–536, 1996
34. STEINHAUSEN M, ENDLICH K: Controversies on glomerular filtration from Ludwig to the present. *Pflügers Arch* 432(Suppl):R73–R81, 1996